CLAIMS

What is claimed is:

1. A method for assaying presence, activity, or both, of an enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the method comprising:
   (a) under conditions which render the enzyme active, reacting the enzyme with a corresponding substrate for a time sufficient to yield phosphorylated product when assaying a kinase or a dephosphorylated product when assaying a phosphatase; and
   (b) contacting the product with a binding matrix; whereby product is fixed to the matrix; and then
   (c) analyzing the matrix for presence of, amount of, or both the presence and the amount of the product fixed to the matrix, whereby the presence, the activity, or both the presence and activity of the enzyme can be determined.

2. The method of Claim 1, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 2.7.1.67, EC 2.7.1.68, and EC 2.7.1.137.

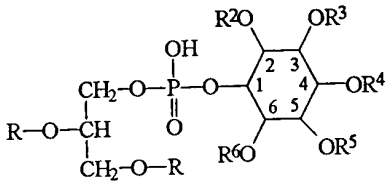
3. The method of Claim 2, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated support.

4. The method of Claim 2, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated regenerated cellulose support.

5. The method of Claim 2, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

6. The method of Claim 5, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phospho-imager.

7. The method of Claim 2, wherein in step (a), the enzyme is reacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously phosphate.

8. The method of Claim 1, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 3.1.3.27, EC 3.1.3.36, EC 3.1.3.64, EC 3.1.3.67, EC 3.1.4.10, and EC 3.1.4.11.

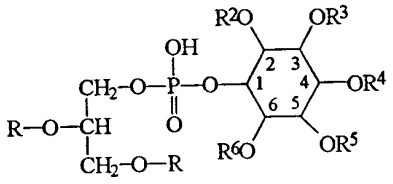
9. The method of Claim 8, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated support.

10. The method of Claim 8, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated regenerated cellulose support.

11. The method of Claim 8, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

12. The method of Claim 11, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phospho-imager.

13. The method of Claim 8, wherein in step (a), the enzyme is reacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously hydrogen.

14. A method for assaying presence, activity, or both, of an enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the method comprising:

(a) under conditions which render the enzyme active, reacting the enzyme with a corresponding substrate, the substrate including a binding moiety, for a time sufficient to yield phosphorylated product when assaying a kinase or a dephosphorylated product when assaying a phosphatase, the product also including the binding moiety; and

(b) contacting the product with a binding matrix specific for the binding moiety; whereby product is specifically fixed to the matrix; and then

(c) analyzing the matrix for presence of, amount of, or both the presence and the amount of the product, whereby the presence, the activity, or both the presence and activity of the enzyme can be determined.

15. The method of Claim 14, wherein the binding moiety is biotin and the binding matrix is avidin or streptavidin immobilized on a solid support.

16. The method of Claim 14, wherein the binding moiety is an antigenic determinant and the binding matrix is an antibody specific for the antigenic determinant, the antibody being immobilized on a solid support.

17. The method of Claim 14, wherein the binding moiety is an antibody, and the binding matrix is an antigenic determinant specific to the antibody, the antigenic determinant being immobilized on a solid support.

18. The method of Claim 14, wherein the binding moiety is an antibody, and the binding matrix is an anti-antibody specific for the antibody, the anti-antibody being immobilized on a solid support.

19. The method of Claim 14, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 2.7.1.67, EC 2.7.1.68, and EC 2.7.1.137.

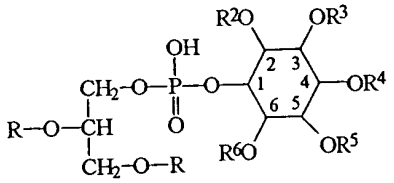
20. The method of Claim 19, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated support.

21. The method of Claim 19, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated regenerated cellulose support.

22. The method of Claim 19, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

23. The method of Claim 22, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phospho-imager.

24. The method of Claim 19, wherein in step (a), the enzyme is reacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously phosphate.

25. The method of Claim 14, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 3.1.3.27, EC 3.1.3.36, EC 3.1.3.64, EC 3.1.3.67, EC 3.1.4.10, and EC 3.1.4.11.

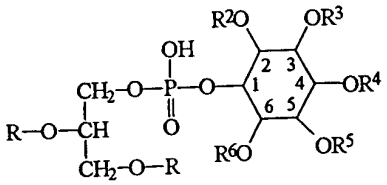
26. The method of Claim 25, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated support.

27. The method of Claim 25, wherein in step (b), the product is contacted with a binding matrix comprising an aldehyde-activated regenerated cellulose support.

28. The method of Claim 25, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

29. The method of Claim 28, wherein in step (a), the enzyme is reacted with the corresponding substrate in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phospho-imager.

30. The method of Claim 25, wherein in step (a), the enzyme is reacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously hydrogen.

31. A method for assaying presence, activity, or both, of an enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the method comprising:

(a) contacting an enzyme substrate specifically reactive with an enzyme of a classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4 with a binding matrix; whereby the enzyme substrate is fixed to the matrix; and then

(b) contacting the substrate fixed to the matrix with an enzyme under conditions wherein the enzyme is active for a time sufficient to yield phosphorylated product fixed to the matrix when assaying a kinase or a dephosphorylated product fixed to the matrix when assaying a phosphatase; and then

(c) analyzing the matrix for presence of, amount of, or both the presence and the amount of the product fixed to the matrix, whereby the presence, the activity, or both the presence and activity of the enzyme can be determined.

32. The method of Claim 31, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 2.7.1.67, EC 2.7.1.68, and EC 2.7.1.137.

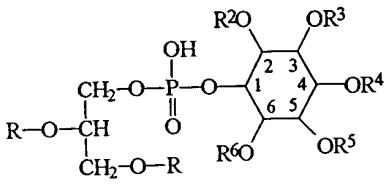
33. The method of Claim 32, wherein in step (a), the enzyme substrate is contacted with a binding matrix comprising an aldehyde-activated support.

34. The method of Claim 32, wherein in step (a), the enzyme is contacted with a binding matrix comprising an aldehyde-activated regenerated cellulose support.

35. The method of Claim 32, wherein in step (b), the substrate is contacted with the enzyme in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

36. The method of Claim 35, wherein in step (b), the substrate is contacted with the enzyme in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phosphorimager.

37. The method of Claim 32, wherein in step (a), the matrix is contacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and

R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously phosphate.

38. The method of Claim 31, wherein the enzyme assayed is classified within an enzyme classification selected from the group consisting of EC 3.1.3.27, EC 3.1.3.36, EC 3.1.3.64, EC 3.1.3.67, EC 3.1.4.10, and EC 3.1.4.11.

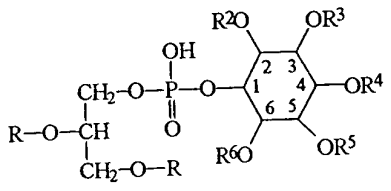
39. The method of Claim 38, wherein in step (a), the substrate is contacted to a binding matrix comprising an aldehyde-activated support.

40. The method of Claim 38, wherein in step (a), the substrate is contacted to a binding matrix comprising an aldehyde-activated regenerated cellulose support.

41. The method of Claim 38, wherein in step (b), the substrate is contacted with the the enzyme in the presence of labeled phosphate groups, and in step (c), the matrix is analyzed by determining the presence, the amount, or the presence and amount, of labeled phosphate groups fixed to the matrix.

42. The method of Claim 41, wherein in step (b), the substrate is contacted with the enzyme in the presence of ^{32}P-labeled phosphate groups, and in step (c), the matrix is analyzed using a scintillation counter or a phospho-imager.

43. The method of Claim 38, wherein in step (a), the matrix is contacted with a substrate of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate, provided that not all of R^2,R^3, R^4, R^5 and R^6 are simultaneously hydrogen.

44. The method of Claim 31, wherein in step (a), the enzyme substrate is contained within a cell lysate and the cell lysate is contacted with the matrix.

45. The method of Claim 31,wherein in step (a), the enzyme substrate is contained within an organic-phase solution and the organic-phase solution is contacted with the matrix.

46. The method of Claim 31, wherein in step (a), the enzyme substrate is contacted with the matrix in the absence of drying the substrate and in the absence of extracting the substrate from an organic phase into an aqueous phase prior to contacting it with the matrix.

47. A kit for assaying presence, activity, or both, of an enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the kit comprising:

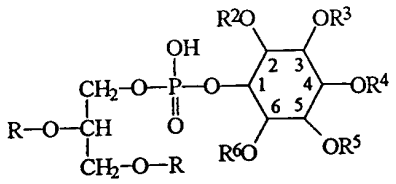
an amount of reaction buffer disposed in a first container;

an amount of substrate for an enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the substrate disposed in a second container;

an amount of binding matrix; and

instructions for use of the kit.

48. The kit of Claim 47, wherein the substrate is of formula:



wherein each R is independently an unsubstituted or substituted C_2 to C_{24} alkyl, alkenyl, alkylcarbonyl, or alkenylcarbonyl group, and R^2,R^3, R^4, R^5 and R^6 are selected from the group consisting of hydrogen and phosphate.

49. The kit of Claim 47, wherein the binding matrix is aldehyde-activated regenerated cellulose.

50. The kit of Claim 47, wherein the substrate further comprises a binding moiety attached thereto.

51. The kit of Claim 50, wherein the binding moiety is biotin.

52. The kit of Claim 50, wherein the binding matrix is avidin or streptavidin immobilized on an inert support.

53. The kit of Claim 47, further comprising an amount of purified enzyme classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4, the enzyme disposed in a third container;